13 THC AND THC-CARBOXYLIC ACID QUANTITATION AND CONFIRMATION IN BLOOD BY LIQUID/LIQUID EXTRACTION AND GCMS ANALYSIS

13.1 Summary

13.1.1 Delta-9-tetrahydrocannabinol (THC), its prinicipal metabolite 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCA) and their deuterated internal standards are extracted from biological samples using an acetonitrile precipitation. After centrifugation, the supernatant acetonitrile layer is made basic and extracted with hexane/ethyl acetate. The organic phase (containing THC) is dried under nitrogen and derivatized with trifluoroacetic anhydride (TFAA) in chloroform, forming the TFA derivatives of THC and deuterated THC. The remaining aqueous phases (containing THCA) are acidified and re-extracted with hexane/ethylacetate, dried under nitrogen and derivatized with BSTFA to form the silyl derivatives of THCA and deuterated THCA. The derivatized samples are injected into the GC/MS for confirmation and quantitation by selected ion monitoring (SIM).

13.2 Specimen Requirements

13.2.1 2.0 mL blood, biological fluid or tissue homogenate.

13.3 Reagents and Standards

13.3.1 Delta 9-THC, 1 mg/mL

13.3.2 9-Carboxy-11-nor-delta 9-THC, 1 mg/mL

13.3.3 Delta 9-THC-d_3, 1 mg/mL

13.3.4 9-Carboxy-11-nor-delta 9-THC-d_3, 1 mg/mL

13.3.5 Sodium hydroxide

13.3.6 Concentrated hydrochloric acid

13.3.7 Methanol

13.3.8 Hexane

13.3.9 Ethyl acetate

13.3.10 Chloroform (with amylene preservative)

13.3.11 Acetonitrile

13.3.12 Trifluoroacetic acid anhydride (TFAA)

13.3.13 BSTFA with 1% TMCS

13.3.14 Heptane

13.4 Solutions, Internal Standards, Calibrators and Controls

13.4.1 0.2 M Sodium hydroxide: Weigh 8 grams NaOH. Transfer to 1 L volumetric flask and QS to volume with dH_2O.

13.4.2 1 N Hydrochloric Acid: Pipet 82.5 mL concentrated HCl into I L volumetric flask and QS to volume with dH_2O.

13.4.3 Hexane:Ethyl Acetate (9:1): Add 450 mL hexane to 50 mL ethyl acetate (v/v). Prepare fresh daily.

13.4.4 Hexane:Ethyl Acetate v/v (1:1): Mix 100 mL hexane with 100 mL ethyl acetate (v/v). Prepare fresh daily.

13.4.5 Working solution A for THC and THCA (2 µg/mL): Pipet 20 µL of 1 mg/mL stock solution of both THC and THCA into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

13.4.6 Working Solution B (0.2 µg/mL): Pipet 1 mL of 2 µg/mL THC/THCA working solution A into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

13.4.7 Quality Control (QC) standard solutions for THC and THCA:

13.4.7.1 10 µg/mL THC QC solution: Pipet 100 µL of 1 mg/mL THC stock (manufacturer or lot number different than that used for calibrators) into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

13.4.7.2 1 µg/mL THC QC solution: Pipet 1 mL 10 µg/mL THC QC solution into 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

13.4.7.3 10 µg/mL THCA QC solution: Pipet 100 µL of 1 mg/mL THCA stock (manufacturer or lot number different than that used for calibrators) into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

13.4.8 Internal standard solutions for THC-d_3 and THCA-d_3

13.4.8.1 10 µg/mL THC-d_3 /THCA-d_3 internal standard mix: Pipet 1 mL of the 100 µg/mL THC-d_3 stock solution and 100 µl of the 1 mg/mL THCA-d_3 stock solution into a 10 mL volumetric flask and QS to volume with methanol. Store in freezer.

13.4.8.2 0.4 µg/mL THC-d_3 /THCA-d_3 working solution: Pipet 1 mL of the 10 µg/mL THC-d_3 /THCA-d_3 internal standard mix into a 25 mL volumetric flask and QS to volume with dH_2O. Prepare fresh daily.

13.4.9 THC and THCA Calibrators: Pipet the following volumes of working standards into 16 x 125 mm tubes to achieve the following calibrator concentrations. Add 2 mL blank blood to each tube.

13.4.9.1 Cal 1: 0.100 mg/L THC and THCA: 100 µL of 2 µg/mL THC/THCA working standard A.

13.4.9.2 Cal 2: 0.050 mg/L THC and THCA: 50 µL of 2 µg/mL THC/THCA working standard A.

13.4.9.3 Cal 3: 0.010 mg/L THC and THCA: 10 µL of 2 µg/mL THC/THCA working standard A.

13.4.9.4 Cal 4: 0.005 mg/L THC and THCA: 50 µL of 0.2 µg/mL THC/THCA working standard B.

13.4.9.5 Cal 5: 0.002 mg/L THC and THCA: 20 µL of 0.2 µg/mL THC/THCA working standard B.

13.4.9.6 Cal 6: 0.001 mg/L THC and THCA: 10 µL of 0.2 µg/mL THC/THCA working standard B.

13.4.10 Controls:

13.4.10.1 0.004 mg/L THC: Pipet 8 µL of 1 µg/mL THC QC Solution into an appropriately labeled 16 x 125 mm screw cap tube containing 2 mL blank blood.

13.4.10.2 0.040 mg/L THCA: Pipet 8 µL of 10 µg/mL THCA QC Solution into an appropriately labeled 16 x 125 mm screw cap tube containing 2 mL blank blood.

13.4.10.3 Negative blood control: Blood bank blood or equivalent previously determined not to contain THC or THCA.

13.5 Apparatus

13.5.1 Agilent GC/MSD, Chemstation software, compatible computer and printer

13.5.2 Screw cap test tubes, 16 x 125 mm

13.5.3 Screw cap test tubes, 13 x 100 mm

13.5.4 Test tube rotator

13.5.5 Centrifuge capable of 2,000-3,000 rpm

13.5.6 Nitrogen evaporator with heating block

13.5.7 Vortex mixer

13.5.8 GC autosampler vials with inserts

13.5.9 GC/MSD Conditions. Instrument conditions may be changed to permit improved performance.

13.5.9.1 Acquisition Mode: SIM

13.5.9.1.1 The following is an example of ions are acquired, with the ions used for quantitation underlined:

THC:	410, 395
THC-d_3:	413, 398
THCA:	371, 473, 488
THCA-d_3	374

13.5.9.2 Column: HP 5MS 25 m x 0.25 mm x 0.25 µm

13.5.9.3 Detector Temperature: 280° C

13.5.9.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 130° C
- Initial time: 1 minutes
- Ramp 16° C/min
- Final Temp 290° C
- Final Time 9 minutes
- Run Time 28 minutes

13.5.9.4.1 Inlet

- Mode: Splitless
- Temperature: 270° C
- Injection volume: 2.0 µL
- Purge Time ON at 1.0 minute

13.6 Procedure

13.6.1 Label clean 16 x 125 mm screw cap tubes-blank, calibrators, controls and case sample IDs.

13.6.2 Prepare calibrators and controls.

13.6.3 Add 2 mL case specimens to the appropriately labeled tubes.

13.6.4 Add 200 µL 0.4 µg/mL THC-d_3 /THCA-d_3 internal standard working solution to each tube. Vortex briefly.

13.6.5 Slowly, add dropwise 2.5 mL cold (freezer temperature) acetonitrile to each tube while vortexing. Continous vortexing, not mere mixing, is essential.

13.6.6 Vortex an additional 30 seconds.

13.6.7 Cap tubes.

13.6.8 Centrifuge at approximately 2800 rpm for 15 minutes.

13.6.9 Place tubes in refrigerator overnight so that the three layers separate: Bottom - blood clot, Middle – serum, Top– acetonitrile

13.6.10 Transfer top (acetonitrile) layer to clean 16 x 125 mL tubes taking care not to transfer any lower layers.

13.6.11 THC Extraction and Derivatization

13.6.11.1 Add 2 mL 0.2 N NaOH to each tube.

13.6.11.2 Add 4 mL freshly prepared 9:1 v/v hexane/ethyl acetate to each tube.

13.6.11.3 Cap tubes and rotate for 30 minutes.

13.6.11.4 Centrifuge at approximately 2800 rpm for 15 minutes.

13.6.11.5 Three layers will form: Bottom – aqueous base, Middle – acetonitrile, Top – hexane/ethyl acetate

13.6.11.6 Transfer top hexane/ethyl acetate layer containing THC to 13 x 100 mm screw cap tubes. **Save lower layers for THCA extraction (13.6.12).**

13.6.11.7 Evaporate hexane/ethyl acetate at approximately 50° C under nitrogen.

13.6.11.8 Add 200 µL chloroform containing amylene preservative to each tube.

13.6.11.9 Add 200 µL trifluoroacetic acid anhydride to each tube.

13.6.11.10 Cap tubes and vortex briefly. Heat samples for 15 minutes at 90° C.

13.6.11.11 Evaporate to dryness at approximately 60° C under nitrogen.

13.6.11.12 Reconstitute with 40 µL heptane. Vortex briefly. Transfer to GC autosampler vials. Inject 2 µL of each sample on GCMS in SIM mode

13.6.12 THCA Extraction and derivitization

13.6.12.1 To lower layers from 13.6.11.6, add 2 mL 1.0 N HCl.

13.6.12.2 Add 4 mL freshly prepared 9:1 v/v hexane/ethyl acetate to each tube.

13.6.12.3 Cap tubes and rotate for 30 minutes.

13.6.12.4 Centrifuge at approximately 2800 rpm for 15 minutes.

13.6.12.5 Transfer top (organic) layer containing THCA to 13 x 100 mm screw cap tubes.

13.6.12.6 Evaporate to dryness at approximately 50° C under nitrogen.

13.6.12.7 Add 50 µL ethyl acetate to each tube.

13.6.12.8 Add 50 µL BSTFA with 1% TMCS to each tube.

13.6.12.9 Cap and vortex each tube. Heat samples for 30 minutes at 60° C.

13.6.12.10 Transfer to GC autosampler vials. Inject 2 µL of each sample on GCMS in SIM mode.

13.7 Calculation

13.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios (using the target ions underlined above) versus calibrator concentration.

13.7.2 Qualifier ion ratio range. The qualifier ion ratio range is calculated by determining the mean ion ratio from all calibrators used in the calibration curve.

13.8 Quality Control

13.8.1 See Toxicology Quality Guidelines

13.9 References

13.9.1 CR Goodall and RJ Basteyns. A reliable method for the detection, confirmation , and quantitation of cannabinoids in blood. *J Anal Tox* 19(6): 419-426, 1995

13.9.2 PM Kemp, IK Abukhalaf, JE Manno, BR Manno, DD Alford and GA Abusada. Cannabinoids in humans: Analysis of 9-THC and six metabolites in plasma and urine using GCMS. *J Anal Tox* 19(5): 285-291, 1995.

13.9.3 Randall Edwards and Terri Woods, in-house development.